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Application Note

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Sterisart®

The Sterisart® Septum Enables Reliable Sampling from a Closed System Sterility Testing Unit

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Abstract

In this study, we evaluated the Sterisart® closed system sterility testing device, with a septum, for the recurrent sterile extraction of samples. The results demonstrate that even after more than 100 repeated septum sampling events, which far exceeds any foreseeable sampling requirements, the septum remains intact and the growth media contained in these canisters remains sterile.

The Sterisart® septum allows easy inoculation and sampling, and enables the coupling of the conventional closed system sterility testing with rapid detection methods.

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Introduction

Pharmaceutical products are routinely manufactured under strict GMP guidelines. Despite these strict codes, as a fail-safe prior to batch release, all pharmaceutical products undergo stringent sterility testing to identify the potential presence of viable microorganisms. It is crucial that pathogenic microbes, such as bacteria, viruses and fungi, are detected in contaminated products before patients come in contact with them. There have been rare instances where compromised drugs have been released to the market with devastating consequences, for the patients and also the pharmaceutical companies.

Sterility tests are performed in accordance with the regulatory requirements defined by the International Pharmacopeia (USP <71>, EP 2.6.1, JP 4:06). Sterility testing can be performed either by direct inoculation | transfer, or membrane filtration, which is the method of choice. Products are tested for sterility by direct inoculation only when the properties of the product do not permit membrane filtration. The membrane filtration approach typically relies on a closed filtration unit containing a membrane with a pore size not greater than 0.45 µm and that has reliably demonstrated the retention of microorganisms. Other components of the system include a suitable pressure supply (such as a peristaltic pump) that drives the sample across the membrane filter, an appropriate membrane rinsing solution, and growth | culture media. This closed setup is conventionally cleanroom compliant to eliminate any contamination risks and consequent false positives. Once sample filtration is complete, the closed system is incubated, typically for 14 days, and screened for turbidity as an indicator of microbial contamination.

Sterisart® canisters are a closed system for sterility testing based on the membrane filtration method. This closed system excludes the need for physically manipulating membrane filters and thereby mitigates the risk of secondary contamination and false positives. However, sample extraction is a prerequisite, when the growth media is rendered turbid by microbial growth, following the prescribed 14 days of incubation. If microbial growth is detected, the identity of the microorganism and the source of the contamination is determined, and the sterility test is declared invalid and then repeated. Aseptic sample withdrawal or aseptic enzyme supplementation, for instance to deactivate antibiotics that might result in false negatives, may also be required after filtration or during incubation.

Precipitation of the filtered test sample, or an adverse color change due to the inherent properties of the compound, can also render the growth media turbid, even prior to incubation at the prescribed temperatures. This convolutes the interpretation of the sterility test and the certification of the batch for release; the batch may require additional testing by sample extraction from the canister and subsequent sub-culturing.

Sample extraction in conventional sterility test systems involves puncturing or cutting the tubing leading to the inlet of the canister and then attempting to carefully extract a sample, without compromising the integrity of the canister or its contents. Sample extraction by cutting the tubing precludes repeated sampling. Multiple sampling using other approaches can increase the risk of contamination by compromising the closed system.

The Sterisart® septum was designed to facilitate repeated sampling during incubation of the growth promotion test. In this report, we show that multiple sampling performed through the Sterisart® canister septum – over 100 times – exceeding any conceivable requirement for aseptic sampling, does not lead to the contamination of the system.

Reasons for septum usage:

a)	The growth media is rendered turbid by microbial growth, following incubation, and necessitates the identification of the micro-organism as part of a root cause analysis.
b)	The product renders the growth medium turbid, prior to incubation, and requires sub-culturing	dilution.
c)	Samples are drawn to test for microbial contamination by rapid detection methods.
d)	Samples are supplemented with agents to counteract anti-microbial components of the tested product.

Materials and Methods

Consumables
Tryptic soy broth (TSB) (Gila/BD), Fluid thioglycollate medium (FTM) (Gila/BD), TSB (Merck), FTM (Merck), Tryptic soy agar (TSA) (Merck), Glass reaction tubes, 30 ml (Borosil), Needle – 0.90 × 70 mm, 20G × 2 ¾ (Sterican – B. Braun), Syringe – F Luer (Omnifix – B. Braun).

Equipment
Sterisart® universal pump, Incubator (Sartorius Stedim Biotech GmbH), Combisart® 3-branch filtration manifold (Sartorius Stedim Biotech GmbH), e.jet Pump (Sartorius Stedim Biotech GmbH).

Sterisart® NF sterility testing system
16467--------GSD, 16475--------GSD, 16466--------GSD

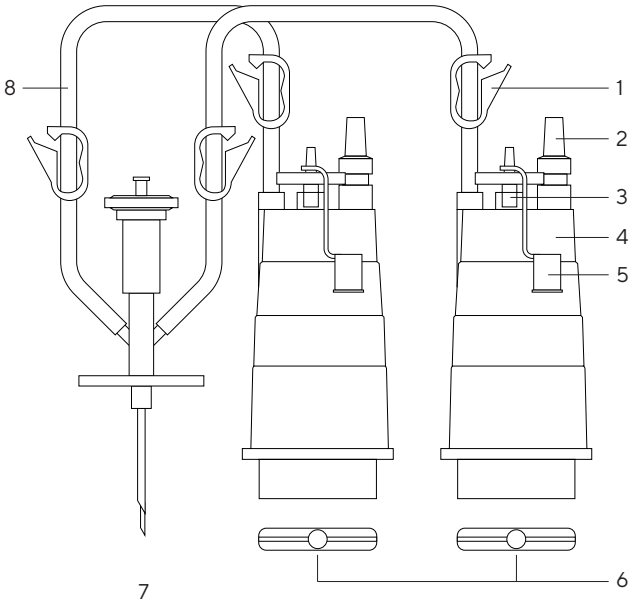
Membrane filtration:
Ten individual Sterisart® canisters from the three types of septum variants (30 in total) were analyzed in the septum sampling tests. One of the ten canisters (from each Sterisart® canister type) served as a negative control (i.e. samples were not extracted from this canister until day 24 of the test).

The Sterisart® canisters were filled with growth media under aseptic sterile conditions in a biosafety cabinet. The two Sterisart® canisters were positioned in the pump holder and the Sterisart® tubing system was thread through the pump head. The outlet of each Sterisart® canister was sealed using the enclosed wing nut plugs. The two sterile vent filters were left uncapped. The yellow tube clamp at the outlet of the Y-distributor was opened and the adjacent white tube clamp closed. The dual-needle metal spike was inserted into a bottle containing FTM and the Sterisart® Universal pump was switched on. The pump was switched off once a predefined volume (75 ml) of medium was transferred into the first canister. The white tube clamp at the outlet of Y-distributor was opened and the adjacent yellow clamp closed. The dual-needle metal spike was inserted into a bottle containing TSB and the Sterisart® Universal pump was switched on. A similar volume (75 ml) of medium was transferred into the second canister. The tubing was sealed off using the two clamps above the inlets of the canisters and the tubing was cut off. Please refer to our **Sterisart® NF gamma user manual** for a pictorial depiction of the described process.

Sterisart® canisters containing TSB, the recommended growth media used in the detection of low incidence fungi and aerobic bacteria, were incubated at 22.5° C for 24 days. Sterisart® canisters containing FTM, the recommended growth media for cultivating aerobic, microaerophilic, and anaerobic microorganisms were incubated at 32.5° C for 24 days.

Septum sampling:
Samples were extracted from the Sterisart® canisters under sterile conditions in a biosafety cabinet. Three samples of 100 µl each were extracted twice a day from the top, middle, and bottom of the Sterisart® canister, over a period of 17 days (3 × 2 × 17 = 102 samples). The extracted samples were transferred into the glass reaction tubes containing the sterile liquid media, FTM and TSB. The vials containing TSB were incubated at 22.5° C for 14 days, and the vials containing FTM were incubated at 32.5° C for 14 days. The results were recorded by photographing each Sterisart® unit and the corresponding extracted sample.

Microbial enumeration:
A final inspection was performed using a black gridded membrane filter placed in a sterile Sartorius Combisart® filtration unit and connected to an e.jet pump. 60 – 70 ml of TSB (following the 24 day incubation period of the Sterisart® canisters) was filled into the funnel and filtered through the black membrane filter. The filter was transferred using sterile forceps onto a TSA plate, and the plate was incubated at 36° C for 3 – 5 days. These plates were then inspected for microbial contamination.



No.	Component
1.	Pre-installed tube clamp
2.	Connector with septum for sterile sampling
3.	Vent filter
4.	Sterisart® container
5.	Tethered filter cap
6.	Wing nut plug
7.	Dual-needle metal spike for closed containers (16466)
8.	Tubing

Results and Discussion

After 102 septum piercings and repeated sample withdrawals, it was established that all Sterisart® canisters (3 × 9 containing FTM, and 3 × 9 containing TSB; the 10th canister containing FTM and TSB serving as their respective controls) were sterile and showed no detectable microbial contamination after 24 days. (Figure 1)

Similarly, the extracted samples were likewise sterile and free of microbial growth demonstrating that the Sterisart® septum promotes efficient and highly reliable aseptic sampling. (Figure 2)

Figure 1: No microbial contamination after repeated sample extraction. Representative images of the Sterisart® 16466 GSD version filled with TSB (A and B) FTM (C and D) incubated for 24 days at 22.5° C and 32.5° C respectively. Negative controls are shown in A and C.
Canisters in B and D were pierced 102 times for sample extraction.

Figure 2: No microbial contamination in samples extracted from Sterisart® 16466 GSD after incubation in glass vials. Samples were inoculated into glass vials containing TSB (upper panel) and FTM (lower panel) were grown at 22.5° C and 32.5° C, respectively, for 14 days.

Coring can occur when a septum has been punctured multiple times or if an inappropriate needle type is used. Only after 36 piercings were small particles observed in some Sterisart® canisters. These particles were collected after 24 days on a black membrane filter and monitored for their ability to form colonies on TSA plates. These particles did not demonstrate any growth even after an incubation period of five days, suggesting that these particles are not biological in nature. Based on their morphology, we conclude that these inert particles are fragments of rubber that are sheared off the septum during repeated piercing with syringe needles. These fragments do not influence the efficacy of the sterility test and are barely visible in the growth medium.

We recommend that septum sampling be performed only after unplugging the sterile vent (i.e. uncapped) in a controlled environment.

Our results demonstrate that Sterisart® canisters remain a closed and sterile unit, even after successive sampling for a tested total of 102 extractions.

Figure 3: Representative image of the Sterisart® septum after 102 sample extractions.

Conclusion

In summary, we demonstrate that the Sterisart® septum is exceedingly robust and maintains an intact sterile environment even after more than a hundred sample extractions.

The presence of preservatives and anti-microbial agents, in products tested for sterility, have to a great extent impeded the adoption of rapid detection methods that rely on direct inoculation. Membrane filtration, and subsequent membrane rinsing, of such products curtails the risk of false negatives during sterility testing. By also facilitating the analysis of large volumes through membrane filtration and by enabling the extraction of samples, we afford our users the ability to integrate closed system sterility testing with rapid sterility testing methods.

However, some slow growing anaerobes can be difficult to detect using some rapid sterility methods. Given that septum sampling does not compromise the integrity of the closed system sterility test, we provide our customers with the potential to sample for rapid sterility testing, yet re-incubate the sterility tests for the stipulated 14-day period of incubation.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Mem
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